REMARKS

Status of the Claims

Claims 1-16, 19-79 have been canceled. New claims 83-104 have been added. Accordingly, claims 17, 18, and 80-104 are pending for consideration before the Patent Office.

The Office Action has indicated that claims 80-82 are allowable.

Amendments to the Claims and the Specification

Line 4 of the paragraph beginning on page 22, line 7 has been amended to replace "titrate" with "citrate". This is an obvious typographical error. Support can be found in line 7 of the same paragraph.

Claim 17 has been amended to more particularly point out and more distinctly claim Applicant's invention.

Claim 18 has been amended to more particularly point out and more distinctly claim Applicant's invention.

Claim 80 has been amended to replace "titrate" in line 5 of the claim with "citrate". This is an obvious typographical error. Support for the amendment can be found in line 8 of the claim.

Support for new claims 83-104 is summarized in the table below. The new claims do not add prohibited new matter.

Claim(s)	Support
83, 84	Original Claim 60
85	Original Claim 61
86	Original Claim 62
87	Original Claim 63

88	Original Claim 64
89	Original Claim 65
90	Original Claim 66
91	Original Claim 69
92	Original Claim 71
93, 94	Original Claim 72
104	Original Claim 73
95, 96	Original Claims 60 and 61; Page 3, lines 6-11; Page 60, lines 7-10
97	Page 51, line 20-23
98-100	Original Claims 74-76
101-103	Original claims 77-79

Objection under 35 U.S.C. § 132

The amendment filed on January 30, 2002, is objected to under 35 U.S.C. § 132 because claims 67, 68, and 70 introduce new matter into the disclosure.

In order to expedite prosecution of the application, Applicant has canceled claims 67, 68, and 70, without prejudice or disclaimer of the subject matter claimed therein.

Rejection under 35 U.S.C. § 112, First Paragraph

Claim 18 is rejected under 35 U.S.C. 112, first paragraph, as not being enabled by the specification for "homologs of SEQ ID NO: 4".

In order to expedite prosecution of the application, Applicant has deleted "conservatively substituted homologs", without prejudice or disclaimer of the subject matter claimed therein.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 17, 18, 64-68, 70, and 72-79 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

In order to expedite prosecution, claims 17 and 18 have been amended to more particularly point out and distinctly claim Applicant's invention. Furthermore, such subject matter is covered in claim 80 and various dependent claims.

Claims 64-68 and 72-79 have been canceled and replaced with new claims that more particularly point out and distinctly claim Applicant's invention.

As acknowledged in the Office Action on page 4, the survivin polypeptides having point mutations are described in the specification on page 63, lines 13-21. On the same page, the specification teaches how to make the mutants. In fact, the positions selected for amino acid substitutions recited in claim 98 are set forth on page 63, lines 13-21. On page 63, lines 22 through page 64, line 2, the specification discloses the functional activity of some of the claimed mutants. Claims 98-100, as they stand, are directed to a polypeptide that differs structurally from the survivin polypeptide having the sequence as set forth in SEQ ID NO: 34 at specific amino acid residues. The structure of the claimed polypeptides of claims 98-100 is based on SEQ ID NO: 34. Accordingly, the specification teaches the detailed structure of the claimed polypeptides. Thus, the specification conveys to the skilled artisan that at the time the application was filed, the inventor had possession of the polypeptides as claimed in claims 98 to 100.

Claims 98-100, as they stand, are not directed to polypeptides having a specific activity. The claims are directed to polypeptides having specific mutations. Given the teachings of the specification of mutant polypeptides, it is within the skill of the artisan to obtain the claimed polypeptides.

Conclusion

In view of the amendments and accompanying remarks, Applicant respectfully requests reconsideration and timely allowance of the pending claims. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact Applicant's undersigned representative to expedite prosecution.

If there are any other fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-0310. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully Submitted,

MORGAN, LEWIS & BOCKIUS LLP

Dated: September 26, 2002

By: _____
Sally P. Teng
Reg. No. 45,397

Customer No. 09629
MORGAN, LEWIS & BOCKIUS LLP
1111 Pennyslvania Avenue, N.W.
Washington, D.C. 20004
Tel.: (202)-739-3000
Fax: (202)-739-3001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 5, line 28 has been amended as follows:

Figures 1A-D show [~~Figure 1 shows~~] the identification of a complementary EPR-1 gene. A, B. Chromosomal location. A digoxigenin-labeled human P1 genomic clone selected by hybridization with the EPR-1 cDNA, was incubated with metaphase chromosomes isolated from phytohemagglutinin-stimulated PBMC in 50% formamide, 10% dextran sulfate and 2X SSC. The EPR-1-hybridizing gene was mapped in single-color labeling to the long arm of a group E chromosome (A[~~, green staining~~]), and in two-color staining with probe D17Z1, specific for the centromere of chromosome 17 (B[~~, red staining~~]), to the long arm of chromosome 17 (B[~~, green staining~~]), to band 17q25. C. Map of the antisense EPR-1 gene. A contig spanning 14796 bp was derived from two EPR-1-hybridizing P1 clones, subcloned in pBSKS$^-$, and completely sequenced on both strands. Orientation of the map is 5'→3' with respect to the position of intron-exon boundaries (see below). Exons are solid boxes, a putative CpG island upstream exon 1 is an open box. The translational initiation codon (ATG) is indicated. Restriction sites are: B, *Bam*HI, H, *Hind*III; P, *Pst*I; S, *Sma*I; X, *Xba*I. D. Intron-exon boundaries of the antisense EPR-1 gene. Positions of the intron-exon boundaries in bp are indicated in parenthesis. The first nucleotide sequence corresponds to SEQ ID NO: 5. The second nucleotide sequence corresponds to SEQ ID NO: 6. The third nucleotide sequence corresponds to SEQ ID NO: 7.

Paragraph beginning on page 6, line 14 has been amended as follows:

Figures 2A-C show [~~Figure 2 shows~~]the complexity and evolutionary conservation of EPR-1-related sequences. A. Southern blot of human genomic DNA. Samples were digested with the indicated restriction enzymes, transferred to GeneScreen nylon membranes and hybridized with the EPR-1 cDNA, in 5X SSC, 0.5% SDS, 5X Denhardt's and 0.1% sodium pyrophosphate at 65∘C. Radioactive bands indicated by an arrow (7.6 kb *Bam*HI, 7.5 kb *Xba*I and *Hind*III fragments of 15, 7.5, 6.4, and 3.7 kb) do not derive from the antisense EPR-1 gene in Figure 1C. B. Southern blot of pulsed field gel electrophoresis. High molecular weight human genomic DNA was digested with the indicated restriction enzymes, separated by pulsed field gel

electrophoresis for 20 h at 200 V with a pulse time of 75 sec, transferred to nylon membrane, and hybridized with the EPR-1 cDNA, as described in A. C. Multiple species Southern blot. *Eco*RI-digested genomic DNA from the indicated species was hybridized with a 3' 548 bp fragment of the EPR-1 cDNA, as described in A. For all panels, molecular weight markers in kb are shown on the left.

Paragraph beginning at page 6, line 28 has been amended as follows:

Figures 3A-F show [~~Figure 3~~]the discordant tissue distribution of sense/antisense EPR-1 transcripts. Northern hybridization was carried out on a multiple tissue adult (**A-C**) or fetal (**D-F**) mRNA blot with single strand-specific probes in 5X SSPE, 10X Denhardt's solution, 2% SDS, 100 mg/ml denatured salmon sperm DNA at 60◦C for 14 h. After washes in 2X SSC at 60◦C and in 0.2X SSC at 22◦C, radioactive bands were visualized by autoradiography. A **and B**. EPR-1-specific single-strand probe. **D and E** [~~B~~]. Antisense EPR-1-specific single-strand probe. C **and F**. Control actin probe. Molecular weight markers in kb are shown on the left.

Paragraph beginning at page 7, line 6 has been amended as follows:

Figures 4A-C show [~~Figure 4 shows~~] the sequence analysis of Survivin and expression in cell lines. A. Predicted translation of the antisense EPR-1 gene product (Survivin)(SEQ ID NO: 34). B. Sequence alignment of the BIR in Survivin (SEQ ID NOS: 8 and 21) and in other IAP proteins by the Clustal method. IAP proteins are identified by accession number, L49433 (SEQ ID NOS: 9 and 22), TNFR2-TRAF signaling complex-associated IAP; L49441 (SEQ ID NOS: 10 and 23), apoptosis 2 inhibitor (Drosophila); P41436 (SEQ ID NOS: 11 and 24), IAP gene from Cydia pomonella granulosis virus; P41437 (SEQ ID NOS 12 and 25), IAP gene from Orgya pseudotsugata nuclear polyhedrosis virus; U19251 (SEQ ID NOS: 13 and 26), NAIP, neuronal inhibitor of apoptosis; U32373 (SEQ ID NOS 14 and 27), IAP-like protein ILP from Drosophila melanogaster; U32974 (SEQ ID NOS: 15 and 28), human IAP-like protein ILP; U36842 (SEQ ID NOS: 16 and 29), mouse inhibitor of apoptosis; U45878 (SEQ ID NOS: 17 and 30), human inhibitor of apoptosis 1; U45879 (SEQ ID NOS: 18 and 31), human inhibitor of apoptosis 2; U45880 (SEQ ID NOS: 19 and 32), X-linked inhibitor of apoptosis; U45881 (SEQ ID NOS: 20 and 33), Drosophila inhibitor of apoptosis. Conserved residues are boxed, identities between Survivin and NAIP (U19251) SEQ ID NOS: 13 and 26 are boxed and shaded. C.

Immunoblotting with anti-Survivin antibody JC700. Protein-normalized aliquots of SDS-extracts of cell lines HEL (erythroleukemia), Daudi and JY (B lymphoma), THP-1 (monocytic), Jurkat and MOLT13 (T leukemia), or non transformed human lung Lu18 fibroblasts, HUVEC or PBMC were separated by electrophoresis on a 5-20% SDS gradient gel, transferred to Immobilon and immunoblotted with control non-immune rabbit IgG (RbIgG), or anti-Survivin antibody JC700 (Survivin). Protein bands were visualized by alkaline phosphatase-conjugated goat anti-rabbit IgG and tetrazolium salts. Molecular weight markers in kDa are shown on the left.

Paragraph beginning at page 7, line 26 has been amended as follows:

Figures 5A-C show [~~Figure 5 shows~~] the regulation of Survivin expression by cell growth/differentiation. HL-60 cells were terminally differentiated to a mature monocytic phenotype by a 72 h culture with 0.1 mM vitamin D_3 plus 17.8 mg/ml indomethacin. Survivin expression before or after vitamin D_3 differentiation was detected by immunoblotting with JC700 antibody, or by Northern hybridization with a Survivin-specific single strand probe. RbIgG, control non-immune rabbit IgG. Protein molecular weight markers in kDa and position of ribosomal bands are shown on the left of each blot.

Paragraph beginning at page 8, line 4 has been amended as follows:

Figures 6A-H show [~~Figure 6 shows~~] the over-expression of Survivin in human cancer, *in vivo*. A. Immunohistochemical staining of human lung adenocarcinoma with affinity-purified anti-Survivin antibody JC700 (20 μg/ml). B. Inhibition of JC700 staining of lung adenocarcinoma by pre-absorption with the immunizing Survivin 3-19 peptide. C. Immunohistochemical expression of Survivin in squamous lung cell carcinoma, but not in the adjacent normal gland epithelium of the lung (C, arrow). D. *In-situ* hybridization of Survivin mRNA in squamous lung cell carcinoma with a Survivin-specific riboprobe. E. Expression of Survivin in pancreatic adenocarcinoma by immunohistochemistry with JC700. F. Normal pancreas, negative for Survivin expression by immunohistochemistry. G. *In situ* hybridization of Survivin mRNA expression in colon adenocarcinoma, but H, not in the adjacent non neoplastic colon gland epithelium (H, arrow). Magnifications are x200, except G, x400.

Paragraph beginning at page 8, line16 has been amended as follows:

Figures 7A-C show [~~Figure 7 shows~~]the effect of Survivin on apoptosis/proliferation. A. EPR-1-regulation of Survivin expression. HeLa cells were transfected with control vector pML1 or the EPR-1 cDNA (which is antisense to Survivin) by electroporation, and selected in hygromicin (0.4 mg/ml). Aliquots of vector control HeLa cells (Vector) or Survivin antisense transfectants (Antisense) were induced with 200 mM $ZnSO_4$ detergent-solubilized, and immunoblotted with the anti-Survivin JC700 antibody. Molecular weight markers are shown on the left. B. Effect of Survivin on apoptosis. Survivin antisense transfectants (1, 2), or vector control HeLa cells (3, 4) were induced with Zn^{2+} ions in 0% FBS for 24 h and stained by the AptoTag method with TdT-catalyzed dUTP labeling of 3'-OH DNA ends and immunoperoxidase (1, 3), or by hematoxylin-eosin (HE) (2, 4). 1. Prominent nuclear DNA fragmentation detected by AptoTag staining in serum-starved Survivin antisense transfectants; 2. HE staining of antisense transfectants reveals the presence of numerous apoptotic bodies (arrows); 3. AptoTag staining of vector control HeLa cells detects a few sparse apoptotic cell (arrow); 4. HE staining of vector control HeLa cells. The arrow indicates a single apoptotic body. Magnification x400. C. Effect of Survivin on cell growth. Twenty thousands vector control HeLa cells (Vector) or Survivin antisense transfectants (Antisense) were seeded in 24-well plates, induced with $ZnSO_4$, harvested at the indicated time points, and cell proliferation was determined microscopically by direct cell count. Data are the mean ± SEM of replicates of a representative experiment out of seven independent determinations.

Paragraph beginning at page 9, line 7 has been amended as follows:

Figures 8A-D show [~~Figure 8 shows~~] the expression of Survivin in HL-60 cells. HL-60 cells were examined via Western and Northern blots for Survivin expression.

Paragraph beginning at page 9, line 12 has been amended as follows:

Figures 10A-H show [~~Figure 10 shows~~]the nucleotide Sequence of *Survivin*, which corresponds to SEQ ID NO: 35. The amino acid sequence displayed in Figure 10 corresponds to SEQ ID NO: 34.

Paragraph beginning at page 9, line 13 has been amended as follows:

Figures 11A-C show [~~Figure 11 shows~~]the expression of Survivin and the generation and characterization of anti-Survivin mAb 8E2 by ELISA and immunoblotting.

Paragraph beginning at page 10, line 3 has been amended as follows:

Figures 14A-B show [~~Figure 14 shows~~] that the presence of Survivin is a negative predictive-prognostic factor in neuroblastoma.

Paragraph beginning at page 10, line 5 has been amended as follows:

Figures 15A-B show [~~Figure 15 shows~~]that the presence of Survivin is a negative predictive prognostic factor in high-grade non-Hodgkin's lymphoma.

Paragraph beginning at page 22, line 7 has been amended as follows:

As used herein, "stringent conditions" are conditions in which hybridization yields a clear and detectable sequence. Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, NaCl, 0.0015 M sodium **citrate** [~~titrate~~], 0.1% SDS at 50°C; or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

In the Claims:

Claim 17 has been amended as follows:

17. (Thrice Amended) An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 34[~~, allelic variants of the polypeptide, and fragments of the polypeptide that retain the ability to inhibit cellular apoptosis~~].

Claim 18 has been amended as follows:

18. (Thrice Amended) **An isolated** [~~A~~] polypeptide comprising the sequence EGWEPDDDPIEEHKKHSSGC (SEQ ID NO: 4)[~~, and its conservatively substituted homologs~~].

Claim 80 has been amended as follows:

80. (Amended) An isolated polypeptide encoded by a nucleic acid molecule, wherein the polypeptide inhibits cellular apoptosis and wherein the nucleic acid molecule hybridizes to the complement of a nucleic acid molecule consisting of nucleotides 2811-2921, 3174-3283, 5158-5275 and 11955-12041 of SEQ ID NO: 35, which encodes SEQ ID NO: 34, under conditions selected from the group consisting of: (1) washing with 0.015 M NaCl, 0.0015 M sodium **citrate** [~~titrate~~], 0.1% $NaDodSO_4$ at 50°C; (2) hybridization in 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; and (3) hybridization in 50% formamide, 5X SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50μg/ml), 0.1 % SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC and 0.1% SDS.